INTERIM ANNUAL REPORT

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FOREWORD

The Interim Annual Report of IARC is designed to update the information provided in the preceding Biennial Report for 1992–1993. However, a number of changes have occurred at the Agency in 1994 that warrant a somewhat fuller introduction than in previous interim reports.

At the personnel level, Dr Armstrong left his position as Deputy Director last December to return to Australia, after several years of excellent service to IARC. In November, Mr Johnson was promoted to Director of Administration and Finance, vacated by the move of Mr Crockett back to WHO headquarters in Geneva. Dr Bartsch, the long-time chief of the Unit of Environmental Carcinogens and Host Factors, accepted the offer of a professorship at the Deutsches Krebsforschungszentrum in Heidelberg, and left the Agency at the beginning of October 1993.

At the scientific level, some restructuring of the laboratories has been undertaken. The Unit of Environmental Carcinogens and Host Factors was dissolved and part of its staff has been incorporated into a new Unit of Endogenous Cancer Risk Factors, under the leadership of Dr Ohshima, in which the main focus is on the role of oxidative stress and chronic inflammation in the evolution of human cancer. A new Unit of Environmental Carcinogenesis, headed by Dr Wild, has been created, which brings together IARC scientists engaged in work on DNA adducts and biomonitoring. In addition, plans are well advanced for the establishment in early 1995 of several other new units that will focus on molecular pathology, genetic cancer susceptibility and genetic epidemiology.

At the support level, considerable progress has been made in improving the Agency's computer equipment, for both scientific and administrative work. A tangible example is the improved appearance of this interim report, produced on the new PC-based word-processing system now installed throughout the building. Other examples are the enhancement of the library's information systems, and fully computerized systems for management of the Agency's finances and its purchases.

More rational use of the space available in the Agency's tower has been achieved by regrouping related activities that had become dispersed during the evolution of the Agency. This will allow better communication and reduce time of travel between floors. Another welcome development that will help in the latter respect is the agreement with the city of Lyons to replace the system of lifts in the tower.

In order to meet the budgetary limitations and redistribute resources in line with new directions of research and new methodologies, the entire budget was closely scrutinized to look

for economies and to see how funds could be transferred towards expansion of the scientific programme. For example, major savings on service contracts for office and laboratory equipment have been achieved. In addition, the conclusion was reached that the Agency could no longer afford the high level of support staff it had previously enjoyed, and a certain number of posts would have to be abolished. However, terms for voluntary separations have been agreed in many cases, thus reducing the potential social hardships. In parallel, natural turnover of scientific staff, although entailing the loss of valued expertise, has provided the opportunity for recruitment of brilliant young scientists both in existing areas of research and in those planned to be developed over the coming year.

In other respects, a significant level of continuity has been ensured, with the majority of the scientific projects carrying on as planned. This report describes in a concise manner the progress made, with cross-reference to the Biennial Report, which provides fuller information on the background to each study and on the investigators and collaborators involved.

We are confident that the experience and reputation of IARC as a whole, the creative spirit of its scientists, and the dedication and expertise of our support staff will ensure that the Agency can successfully expand into new promising research areas and continue making unique contributions to understanding the etiology and possibilities for prevention of human cancer.

P. Kleihues
Director

PART 1. COLLECTION , DISSEMINATION AND ANALYSIS OF DATA ON CANCER FREQUENCY AND IMPACT

1.1 Description of cancer incidence and mortality

1.1.1 *Cancer Incidence in Five Continents* (pp. 1–2 of 1992/93 Biennial Report)

The methodology designed for condensing the data from volumes V and VI on diskette was extended to add the data from the first four volumes of *Cancer Incidence in Five Continents* onto diskette. This makes the full set of data (covering more than 30 years for some parts of the world) readily available for users.

The programs for performing validity checks to improve quality and comparability of published data were reviewed, modified and made available on diskette to cancer registries along with the monograph *Comparability and Quality Control in Cancer Registration* (see Section 1.4.4).

1.1.2 European cancer incidence and mortality database (EUROCIM) (p. 2 of 1992/93 Biennial Report)

Maintenance of a database of cancer incidence and mortality data from European countries is one of the key activities of the European Network of Cancer Registries (see Section 1.4.2). The aims of the project are to validate and standardize data submitted by registries, to distribute a pooled database to the contributing registries, and to provide computer software. Version 1.1 of the EUROCIM software package has now been distributed to 68 registries. A three-day course in March on uses of EUROCIM was attended by 22 participants from cancer registries in eight countries. An update of the EUROCIM database and revised manual are in preparation for release in early 1995.

1.1.3 Analysis of data from collaborating cancer registries (pp. 2–4 of 1992/93 Biennial Report)

Collaborators in cancer registries around the world are assisted in the analysis and presentation of their results, either for local purposes, or in international publications.

The monograph *Cancer in the African Population of Bulawayo, Zimbabwe 1963–1977* (Skinner *et al.*, 1993) was published, and a paper summarizing the major findings is in press (Parkin *et al.*, 1994). Work on analysing and presenting the results for the more interesting cancer sites (bladder, oesophagus, female cancers) was begun (Vizcaino *et al.*, 1994).

IARC Technical Reports on cancer in Thailand (Vatanasapt *et al.*, 1993) and on occupational risk factors for cancer in Tianjin, China (Wang *et al.*, 1994a) were completed, as was a second monograph on cancer in the Philippines, sponsored by UICC (Laudico *et al.*, 1993). The data from the first three years of the cancer registry in Hanoi, Viet Nam, were published (Pham *et al.*, 1993) and a visiting fellow analysed the first data from the registry in Ho Chi Minh Ville.

The database accumulated from various collaborative projects with cancer registries was used to study the epidemiology of lung cancer and liver cancer for certain specific cellular types (Parkin & Sankaranarayanan, 1994; Parkin *et al.*, 1993a).

Material from cancer registries in several developing countries was used to study time trends of major cancers such as lung, stomach, large bowel, cervix uteri and breast (Boffetta & Parkin, 1994; Parkin *et al.*, 1994).

1.1.4 Worldwide burden of cancer (pp. 4–5 of 1992/93 Biennial Report)

The measures of the cancer burden are being extended to other indexes such as prevalence and years of life lost. Prevalence (the number of individuals alive in a population who had a diagnosis of cancer within a defined period) has been estimated for the 18 cancer sites for which incidence and mortality estimates were previously obtained, and for every country in the developed areas.

The proportion of all cancers attributable to tobacco smoking has been estimated as 15%, or 1.1 million new cases per year (25% in men and 4% in women). In developing countries, the etiological fraction is 10%, compared to the 16% estimated for 'western' countries. A preliminary estimate of the etiological fraction due to infectious agents has also been prepared: 22% of all new cancer cases are due to infection with viruses, parasites or bacteria in developing countries; the corresponding figure in developed areas is estimated as 9% (Parkin *et al.*, 1993; Pisani *et al.*, 1993b).

1.1.5 Cancer incidence and mortality maps (p. 6 of 1992/93 Biennial Report)

The preparation of the *Atlas of Cancer Mortality in Central Europe* is now complete, with publication foreseen in the first half of 1995.

Availability of data is being investigated for an updating and expansion of *the Atlas of Cancer Mortality in the EEC* published in 1992 as well as the atlas of central Europe described above, so as to cover the whole European continent for the period 1988–92.

Work on the Mortality and Incidence Cancer Atlas for the former Soviet Union is progressing, with the assistance of Dr Tamara Men in Moscow and of Ms Regina Winkelmann at IARC.

1.1.6 **Studies of survival from cancer** (p. 7 of 1992/93 Biennial Report)

The first part of the concerted action financed by the European Union is now completed and the results of this study are in press as IARC Scientific Publications No. 132. The working group is pursuing its efforts in two directions: (i) a deeper analysis of survival data for specific sites using the existing database; (ii) the collection and analysis of detailed data on stage, diagnostic methods and treatment. This second arm of the study is in particular aimed at understanding the sizable difference in survival observed between European countries.

Population-based survival rates for different cancers are not generally available for most cancers in developing countries, due to difficulties in obtaining adequate follow-up information, and problems in registration. Work has begun on a project to describe the various methods used to achieve satisfactory follow-up information, and to present comparable survival rates for the major cancers for several countries.

1.1 IARC staff publications

Boffetta, P. & Parkin, D.M. (1994) Cancer in developing countries. *CA Cancer J. Clin.* **44**, 81–90

Laudico, A.V., Esteban, D.B. Ngelangel, C.A., Reyes, L.M., Parkin, D.M. & Olivier, S. (1993) *Cancer in the Philippines*, Vol. II., Manila, Philippines Cancer Society Inc.

Parkin, D.M. (1994) Cancer in developing countries. In: Doll, R., Fraumeni, J.F. & Muir, C.S., eds, Trends in cancer incidence and mortality. *Cancer Surveys*, **19/20**, 519–561

Parkin, D.M. & Sankaranaraynan, R. (1994) Overview on small cell lung cancer in the world: industrialized countries, third world, eastern Europe. *Anticancer Res.*, **14**, 277–282

Parkin, D.M., Ohshima, H., Srivatanakul, P. & Vatanasapt, V. (1993a) Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. *Cancer Epidemiol.Biomarkers Prev.*, **2**, 537–544

Parkin, D.M., Pisani, P. & Ferlay, J. (1993b) Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int. J. Cancer*, **54**, 594–606

Parkin, D.M., Vizcaino, A.P., Skinner, M.E.G. & Ndhlovu, A. (1994) Cancer patterns and risk factors in the African population of southwestern Zimbabwe, 1963–1977. *Cancer Epidemiol. Biomarkers Prev.*, **3** (in press)

Pham Thi Hoang Anh, Parkin, D.M., Nguyen Thi Anh & Nguyen Ba Duc (1993) Cancer in the population of Hanoi, Vietnam, 1988–1990. *Br. J. Cancer*, **68**, 1236–1242

Pisani, P., Parkin, D.M. & Ferlay, J. (1993) Estimates of the worldwide mortality from eighteen major cancers in 1985. Implications for prevention, and projections of future burden. *Int. J. Cancer*, **55**, 1–13

Skinner, M.E.G., Parkin, D.M., Vizcaino, A.P. & Ndhlovu, A. (1993) *Cancer in the African Population of Bulawayo, Zimbabwe, 1963–1977: Incidence, Time Trends and Risk Factors* (IARC Technical Reports No. 15) Lyon, IARC

Vatanasapt, V., Martin, N., Sriplung, H., Chindavijak, K., Sontipong, S. Sriamporn, S., Parkin, D.M. & Ferlay, J. (1993) *Cancer in Thailand 1988–1991* (IARC Technical Reports No. 16), Lyon, IARC

Vizcaino, A.P., Parkin, D.M., Boffetta, P. & Skinner M.E.G. (1994) The epidemiology and risk factors for bladder cancer in Bulawayo, Zimbabwe. *Cancer Causes Control* (in press)

Wang, Qing-sheng, Boffetta, P., Kogevinas, M. & Parkin, D.M. (1994a) Cancer Incidence by Occupation and Industry in Tianjin, China 1981–1987 (IARC Technical Reports No. 22), Lyon, IARC (in press)

Wang, Qing-sheng, Boffetta, P., Parkin, D.M. & Kogevinas, M. (1994b) Occupational risk factors for lung cancer in Tianjin, China. *Am. J. Industr. Med.* (in press)

1.2 Description of cancer incidence and of mortality in relation to particular population characteristics or risk factors

1.2.1 Cancer incidence and mortality in migrant populations (p. 9 of 1992/93 Biennial Report)

Collaborative projects include studies of Italian migrants (Geddes *et al.*, 1994), Polish migrants (Tyczynski *et al.*, 1994), and migrants to France (Bouchardy *et al.*, 1994). The emphasis has been either upon hitherto unstudied groups, or on exploiting data sets which permit analysis of trends in risk by time since migration or comparisons between first-generation migrants and their offspring.

Mortality data from Canada are being used to compare risks in migrants from different countries, and in their offspring, with the risk in the local-born population.

Data from the Israel Cancer Registry are being analysed with respect to cancer risks in Jewish migrants to Israel, the offspring of such migrants, and the 'third generation' of Israelis (born in Israel, of Israel-born parents). For technical reasons, this analysis is confined to cancers in the age-group 0–29 years, and thus concentrates upon tumours common in children (e.g., leukaemias and lymphomas), in adolescents (bone tumours, soft tissue sarcomas), or in young adults (carcinomas of nasopharynx, thyroid, ovary and breast).

1.2.2 European Childhood Leukaemia/Lymphoma Incidence Study (ECLIS) (p. 10 of 1992/93 Biennial Report)

This project, started in 1988, is supported by the Radiation Protection Research Action of the European Commission. The objective is to determine whether any change in incidence of childhood leukaemia in Europe which may be observable since 1985 is quantitatively associated with the estimated exposure to radiation due to the Chernobyl accident in April 1986 (Parkin *et al.*, 1993).

Data collection was completed for collaborating centres in 21 countries, and a report prepared. There was little indication after 4½ years of follow-up that any of the trends in incidence can be ascribed to excess radiation from the accident. Potential new participating centres have been identified in Romania and Greece.

1.2.3 **Monitoring of trends in incidence of cancers related to infection with HIV** (p. 11 of 1992/93 Biennial Report)

Data sets from cancer registries, particularly in Europe and Africa, have been updated to permit a first analysis of the time trends in incidence of Kaposi's sarcoma, in relation to the notification rate of AIDS.

1.2.4 **Other ecological studies** (pp. 11 of 1992/93 Biennial Report)

A systematic search for results from international surveys on women's reproductive habits has been undertaken. The data available will be correlated with the mortality and incidence, when possible, of cancers of the breast, uterus and ovary.

Data on the prevalence of infection with some parasites of the urinary and gastrointestinal apparatus are being gathered in collaboration with scientists of other departments in WHO.

1.2 IARC staff publications

Bouchardy, C., Parkin, D.M. & Khlat, M. (1994) Cancer mortality among Chinese and south-east Asian migrants in France. *Int. J. Cancer*, **58**, 1–6

Geddes, M., Balzi, D., Buiatti, E., Brancker, A. & Parkin, D.M. (1994) Cancer mortality among Italian migrants to Canada. *Tumori*, **80**,19–23

Parkin, D.M. & 36 others (1993) Childhood leukaemia following the Chernobyl accident: the European Childhood Leukaemia–Lymphoma Incidence Study (ECLIS). *Eur. J. Cancer*, **29A**, 87–95

Tyczynski, J., Tarkowski, W., Parkin, D.M. & Zatonski, W. (1994) Cancer mortality among Polish migrants to Australia. *Eur. J. Cancer*, **30A**, 478–484

Wabinga, H.R., Parkin, D.M., Wabwire-Mangen, F. and Mugerwa, J.W. (1993) Cancer in Kampala, Uganda, in 1989–91: Changes in incidence in the era of AIDS. *Int. J. Cancer*, **54**, 26–36

1.3 Descriptive studies of childhood cancer

1.3.1 **Descriptive studies of particular types of childhood cancer** (pp. 12–14 of 1992/93 Biennial Report)

The database prepared for the first volume of *International Incidence of Childhood Cancer* (Parkin *et al.*, 1988) has been used to prepare a series of reviews of the descriptive epidemiology of specific cancers (Stiller & Nectoux, 1994; Stiller & Parkin, 1994; Stiller, 1994). A review of the descriptive epidemiology of childhood cancer in developing countries has been prepared (Parkin & Stiller, 1994).

The preparation of the second volume of *International Incidence of Childhood Cancer* was begun, with a first editorial meeting and contact with potential participants. The classification system for childhood cancer was revised to take account of the second edition of the International Classification of Diseases for Oncology (ICD-O II), together with minor modifications.

1.3.2 Neonatal tumours (p. 14 of 1992/93 Biennial Report)

Various sources of data on the occurrence of neonatal tumours in the Rhône region have been explored. Cases are found in traditional sources such as general population or paediatric cancer registries, but many may also be registered solely in population-based registries of congenital malformations (Sasco *et al.*, 1993, 1994a). The high frequency and the poorly understood etiology of neonatal angiomas have led us to set up a case–control study among newborns in public obstetric units in Lyon to evaluate the role of maternal exposures during pregnancy.

1.3 IARC staff publications

Parkin, D.M. & Stiller, C.A. (1994) Childhood cancer in developing countries - environmental factors. *Int. J. Paediat. Hematol./Oncol.* (in press)

Parkin, D.M., Stiller, C.A., Draper, G.J., Bieber, C.A., Terracini, B. & Young, J.L., eds (1988) *International Incidence of Childhood Cancer* (IARC Scientific Publications No. 87), Lyon, IARC

Sasco, A.J., Satgé, D. & Little, J. (1993) Neonatal cancers and drug use during pregnancy. The role of registries of congenital malformations and cancer registries. *Eur. J. Epidemiol.*, **9**, 116–117

Sasco, A.J., Chatard, O., Robert, E., Frappaz, D. & Magnani, C. (1994a) L'enregistrement des tumeurs chez l'enfant de moins de 1 an. In: *Epidémiologie du cancer dans les pays de langue latine* (IARC Technical Reports No. 20), Lyon, IARC, pp. 181–194

Sasco, A.J., Lutz, P., Satgé, D., Pinget, M., Born, A. &, Becmeur, F. (1994b) Treatment for male sterility and neonatal neuroblastoma in the offspring. *Br. Med. J.*, 309, 410

Stiller, C.A. & Nectoux, J. (1994) International incidence of childhood brain and spinal tumours. *Int. J. Cancer*, **23**, 458–464

Stiller, C.A. & Parkin, D.M. (1994) International variations in the incidence of childhood soft-tissue sarcomas. *Paediat. Perinatal Epidemiol.*, **8**, 107–119

Other articles cited

Stiller, C.A. (1994) International variations in the incidence of childhood carcinomas. *Cancer Epidemiol. Biomarkers Prev.*, **3**, 305–310

1.4 Support to cancer registries

1.4.1 International Association of Cancer Registries (pp. 14–15 of 1992/93 Biennial Report)

The International Association of Cancer Registries now has 371 members in 97 countries. The Agency continues to provide a secretariat for the Association. Members represent the Association at most of WHO's meetings, both in Geneva and in the Regions.

The Association has been active in lobbying the EU concerning proposed legislation on data protection, and WHO concerning its decision to modify the widely used 'standard' populations.

The 1993 annual scientific meeting was held in Bratislava, Slovakia. One day was devoted to a symposium on cancer in the ageing, sponsored by the US National Institute of Ageing.

The Association maintains an active publishing programme, in collaboration with IARC (see Section 1.4.4).

1.4.2 European Network of Cancer Registries (pp. 15–16 of 1992/93 Biennial Report)

The European Network of Cancer Registries is funded by the European Union 'Europe Against Cancer' programme. The main aims of the Network are to improve the quality and comparability of cancer registry data in Europe and to make these more easily and rapidly available.

The standardization of cancer registration methods is being promoted by (1) surveys of coding and classification, which have revealed important variations in practice; (2) expert working groups with responsibility for setting standards to reduce these variations; (3) fellowships, which allow staff of Network registries to make short training visits to other registries; (4) consultancy, through which registries can seek expert advice on local technical issues; and (5) courses on cancer registration methods, the first of which was held in Copenhagen in January 1994 with 15 participants from nine countries. A standard set of training materials has been prepared and will be translated into Spanish for a course in Granada.

Information about cancer in Europe is disseminated through EUROCIM (see Section 1.1.2) and printed publications. *Facts and Figures of Cancer in the European Community* has been published and more detailed analysis of cancer in Europe is in progress.

1.4.3 Cancer registration and cancer epidemiology in Latin countries (p. 16 of 1992/93 Biennial Report)

IARC provides support to the "Groupe pour l'Epidémiologie du Cancer dans les Pays de Langue Latine", in particular by contributing to the organization of the annual meeting, especially its associated methodological workshops, and by publishing the proceedings of this meeting as IARC Technical Reports. The 1994 meeting was held at the School of Public Health in Granada, Spain, at the invitation of Dr Carmen Martinez, and was preceded by a methodological workshop on the interpretation and estimation of prevalence. The book on statistical methods in descriptive epidemiology, which was initially based on these seminars, is now available in English (Estève *et al.*, 1994).

1.4.4 Reliability and validity of cancer registry data (pp. 16–17 of 1992/93 Biennial Report)

Comparability and quality control in cancer registration

In collaboration with the International Association of Cancer Registries (IACR), a practical guide on *Comparability and Quality Control in Cancer Registration* (Parkin *et al.*, 1994) has been produced presenting the techniques available to ensure completeness of coverage of the population in the registration area, and to check the accuracy and detail of the information collected. A diskette of the IARC-CHECK program, originally designed to check data contributed to Volume VI of *Cancer Incidence in Five Continents*, is included.

Coding and classification

For both of the main classification systems used in cancer registries (the International Classification of Diseases and the International Classification of Diseases for Oncology), new revisions (the 10th and 2nd respectively) were published recently. In order to allow comparison of data collected using these and earlier versions, a personal computer diskette containing six conversion programs and a user's guide (Ferlay, 1994) was prepared and sent to all members of the International Association of Cancer Registries.

Multiple primaries

Comparison of incidence rates from different centres requires that a standard methodology is used to distinguish second cancers in a single individual from the spread or recurrence of the initial tumour. A set of rules for use in international studies has been agreed with the International Association of Cancer Registries. These have been revised to take account of the second edition of ICD-O, and published as an IARC Internal Report.

1.4.5 Computer software for cancer registries (pp. 17–18 of 1992/93 Biennial Report)

The CANREG set of microcomputer programs for cancer registration in population-based registries is now available in several languages (English, French and Spanish, but also Turkish and Thai systems are used). It has been supplied to many registries in developing countries and to several in Europe. At the present time, about 50 registries are using CANREG. New requests have been received in 1994 from:

Africa: Niamey (Niger), Conakry (Guinea) and Abidjan (Côte d'Ivoire)

Asia: Davao (Philippines), Ho Chi Minh Ville (Viet Nam), Kuala Lumpur (Malaysia), Trivandrum (India)

Americas: Concordia (Argentina), Lima (Peru), Kingston (Jamaica) and Trinidad.

Either registry personnel have visited IARC for a period of training or for data processing, or an IARC staff member has visited registries to install or modify the CANREG system and train the staff.

1.4.6 Other support to cancer registries (pp. 18–22 of 1992/93 Biennial Report)

The Descriptive Epidemiology Programme provides advice on methods for cancer registration, and data analysis, as well as supplying appropriate materials. Cancer registry personnel from Bangkok (Thailand), Blantyre (Malawi), Bujumbura (Burundi), Niamey (Niger), Butare (Rwanda), Conakry (Guinea), Kampala (Uganda), Karachi (Pakistan), and Tehran (Iran) attended IARC to receive basic training in methodology, including visits to collaborating centres in France or Great Britain. During 1993/94, consultant visits were made to cancer registries in Argentina, Bahrain, Brazil, Costa Rica, Cuba, Ecuador, Jamaica, Peru and Uruguay.

Several cancer registries in developing countries receive direct support in the form of a collaborative research agreement, to enable them to start activities or to purchase equipment.

Unit staff participated in a seminar on cancer registration in the Eastern Mediterranean Region, and provided advice on registration at a conference on cancer control in Bangladesh.

1.4 IARC staff publications

Estève, J., Kricker, A., Ferlay, J. & Parkin, D.M. (1993) *Facts and Figures of Cancer in the European Community*, Lyon, IARC

Estève, J., Benhamou, E. & Raymond, L. (1994) *Statistical Methods in Cancer Epidemiology*, Vol. IV, *Descriptive Epidemiology* (IARC Scientific Publications No. 128), Lyon, IARC

Ferlay, J. (1994) *ICD Conversion Programs for Cancer* (IARC Technical Report No. 21), International Agency for Research on Cancer, Lyon

Parkin, D.M., Chen, W.W., Ferlay, J., Galceran, J., Storm, H.H. & Whelan, S.L. (1994) *Comparability and Quality Control in Cancer Registration* (IARC Technical Report No. 19), International Agency for Research on Cancer, Lyon

PART 2. IDENTIFICATION, ELUCIDATION AND EVALUATION OF ENVIRONMENTAL CAUSES OF CANCER

2.1 Evaluation of carcinogenic risks to humans

2.1.1 *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*

In the period covered by this report, four meetings were convened.

2.1.1.1 *Quantitative estimation and prediction of cancer risks (18–22 October 1993)*

A small group of external advisers prepared background documents for a workshop on the scientific principles of quantitative estimation (QEP) and prediction of carcinogenic risks to humans, held in Lyon on 18–22 October 1993. The purpose of the workshop was to review critically the scientific principles of QEP and to advise IARC about its future role in this area.

The main recommendation of the workshop participants was that the primary function of the IARC Monographs programme should remain the identification of carcinogenic hazards to humans. They recommended that although the monographs should not include formal QEP, the presentation of quantitative data on exposure to carcinogens and on the effects of carcinogens should be enhanced. The participants identified a number of activities that the IARC could conduct in areas of analysis, research, education and training in the area of QEP, including other IARC publications.

An extensive summary of the workshop conclusions is available as IARC Internal Report No. 94/004. The background papers are being expanded for publication in the IARC Scientific Publications series.

2.1.1.2 *Ad-hoc Working Group to select priorities for evaluation in the IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (7–9 December 1993)*

At approximately five-year intervals the IARC selects chemicals, groups of chemicals, mixtures, physical and biological agents and exposure circumstances for evaluation in the Monographs series on the basis of advice from an international group of scientists. The fourth priorities working group was convened to discuss lists of 189 agents and exposures nominated by scientists and national institutes from several countries and to assign priorities for consideration or re-consideration within Monographs planned for the period 1995–2000. A total of 91 agents were given high priority. The final report was published as IARC Internal Report No. 93/005.

2.1.1.3 *Some industrial chemicals (Volume 60, 15–22 February 1994)*

A working group convened by IARC evaluated the carcinogenic risks to humans of 14 chemicals. Several of these chemicals are characterized by unsaturated bonds, which are important for their extensive use in the production of polymers and copolymers. Epoxides which can be formed during metabolic reactions at these bonds were also evaluated. Two of the chemicals (ethylene and isoprene) occur in human breath as a result of endogenous formation.

On the basis of limited human and sufficient experimental evidence of carcinogenesis as well as strong supporting genetic evidence, ethylene oxide exposure was judged to be causally associated with human lymphatic and haematopoietic system malignancies (Group 1). For all other chemicals, the human cancer evidence was inadequate for any judgement to be made. Acrylamide and styrene-7,8-oxide were evaluated as probably carcinogenic to humans (Group 2A), on the basis of sufficient experimental evidence of carcinogenicity supported by strong genetic evidence. Isoprene, propylene oxide, 4-vinylcyclohexene and 4-vinylcyclohexene diepoxide were evaluated as possibly carcinogenic to humans (Group 2B) on the basis of sufficient experimental evidence of carcinogenicity. Although for styrene the experimental evidence was only limited, it too was evaluated as a possible human carcinogen, mainly on the basis of metabolic evidence of its conversion to styrene-7,8-oxide. The other six chemicals (2-ethylhexyl acrylate, *N*-methylolacrylamide, ethylene, propylene, methyl methacrylate and vinyl toluene) were not classifiable as to their carcinogenicity to humans (Group 3).

2.1.1.4 Schistosomes, liver flukes and Helicobacter pylori *(Volume 61, 7–14 June 1994)*

It is estimated that about half of the world's population is infected with *Helicobacter pylori*, and there is evidence that this infection is the cause of most cases of chronic gastritis. The Working Group concluded that *H. pylori* plays a causal role in gastric carcinogenesis and reached the evaluation that infection with *H. pylori* is carcinogenic to humans (Group 1).

Infections with three species of schistosomes were evaluated. *Schistosoma haematobium* infection was judged to be causally associated with cancer of the urinary bladder: infection with *S. haematobium* is carcinogenic to humans (Group 1).

Infections with *S. japonicum* and *S. mansoni* have been linked with cancer of the liver and the gastrointestinal tract but the Working Group found the evidence to be less strong: infection with *S. japonicum* is possibly carcinogenic to humans (Group 2B) and infection with *S. mansoni* is not classifiable as to its carcinogenicity to humans (Group 3).

Associations between infections with liver flukes and cholangiocarcinoma have been suggested. The Working Group's evaluation was that infection with *Opisthorchis viverrini* is carcinogenic to humans (Group 1), infection with *Clonorchis sinensis* is probably carcinogenic to humans (Group 2A) while infection with *O. felinius* is not classifiable as to its carcinogenicity to humans (Group 3).

2.1.2 International Network of Carcinogenicity Testing (p. 29 of 1992/93 Biennial Report)

The International Network of Carcinogenicity Testing comprises a small number of laboratories undertaking long-term carcinogenicity studies in experimental animals on a number of priority chemicals. The chemicals or agents are often identified in the Monographs evaluation procedures. At present, four laboratories in Hungary, Russia and Sweden are undertaking tests on extremely low-frequency electromagnetic fields, acetochlor and simazine, trichlorfon and sulfuric acid mists.

2.1 IARC staff publications

McGregor, D.B. (1993) Carcinogenicity and genotoxic carcinogens. In: Ballantyne, B., Marrs, T.C. & Turner, P., eds, *Textbook of Basic and Applied Toxicology*, London, Macmillan, pp. 957–977

McGregor, D.B., Pangrekar, J., Rosenkranz, H.S. & Klopman, G. (1994) A reexamination of the low prevalence of carcinogens in an early carcinogen screen. *Regul. Toxicol. Pharmacol.*, **19**, 97–105

Møller, H., Heseltine, E. & Vainio, H. (1994) Meeting report: Quantitative estimation and prediction of risk at the International Agency for Research on Cancer. *Cancer Res.*, **54**, 3625–3627

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2.2 Occupational causes of cancer

2.2.1 Occupational cancer in industrialized countries

2.2.1.1 *International Register of Workers Exposed to Phenoxy Acid Herbicides and Contaminants* (p. 31 of 1992/93 Biennial Report)

The first mortality follow-up of production workers and sprayers exposed to chlorophenoxy herbicides, chlorophenols and contaminants (principally dioxins and dibenzofurans) has been completed. An excess risk was observed for soft-tissue sarcoma, while no excess was observed for non-Hodgkin lymphoma. Risks appeared elevated for cancers of the thyroid, testis, other endocrine glands, nose and nasal cavity, based on small numbers of deaths. Two nested case–control studies on soft-tissue sarcoma and non-Hodgkin lymphoma have also been completed. Associations were observed between both types of neoplasms and estimated exposure to dioxins and furans, TCDD, and major classes of phenoxy herbicides, but were stronger for soft-tissue sarcomas. An update of the follow-up is in progress.

2.2.1.2 *Cohort study on workers exposed to styrene* (p. 31 of 1992/93 Biennial Report)

An historical cohort study of about 25 000 workers exposed to styrene in the reinforced plastics industry is being conducted in six European countries (Denmark, Finland, Italy, Norway, Sweden, UK). In order to provide quantitative estimates of exposure, an exposure matrix for styrene has been constructed, based on environmental and biological monitoring data. Among exposed workers, no excess was observed for mortality from all causes, from major epithelial cancers, or from neoplasms of the lymphatic and haematopoietic tissues. The rate of mortality from leukaemias and lymphomas increased with time since first exposure. Among subjects exposed for more than one year, a two-fold risk was observed 20 years after first exposure.

2.2.1.3 *Historical prospective study of workers employed in the man-made mineral fibre industry* (p. 32 of 1992/93 Biennial Report)

An historical cohort study was established in 1976 in seven European countries (Denmark, Finland, Germany, Italy, Norway, Sweden, United Kingdom), including workers employed in 13 plants producing rock/slag wool, glass wool or continuous glass filament. Mortality and cancer incidence has been investigated up to 1990–91. An excess risk of lung

cancer was found among rock/slag wool workers, in particular among those first employed more than 20 years before diagnosis. The pilot phase of a case–control study on lung cancer in the rock/slag wool subcohort has indicated the feasibility of a full-scale case–control study to be carried out in 1995–96.

A mathematical model of past fibre exposure has been applied to lung cancer risk among rock/slag wool workers; a non-significant dose–response relationship has been observed with estimated cumulative fibre exposure.

2.2.1.4 *International cohort study on cancer risk among workers in the pulp and paper industry* (p. 33 of 1992/93 Biennial Report)

The historical cohort study of pulp and paper mill workers in Brazil, Canada (eight provinces), Denmark, Finland, France, Germany, Italy, Netherlands, New Zealand, Norway, Poland, South Africa, Spain, Sweden, Switzerland, the UK and the USA is enrolling 150 000 subjects. An industrial hygiene investigation involves the administration of factory exposure questionnaires, examination of production and other industry records and examination of information in exposure data-banks. The study has been completed in Finland, Poland, Spain and the USA. The first combined results are expected in 1996.

2.2.1.5 *International study of cancer risk in biology research laboratory workers* (pp. 33–34 of 1992/93 Biennial Report)

The retrospective cohort study to assess mortality among workers in public research institutions of eight European countries is progressing (Sasco, 1994a,b). Methodological work is being conducted to construct 'scientific activity–exposure' matrices to be used in analysing the effect of exposures on cancer risk (Sasco, 1993; Sasco *et al.*, 1994). An ancillary project, the EVIL programme, has been set up to evaluate exposure to selected viruses in the research population.

2.2.1.6 *International collaborative study on workers exposed to lead* (p. 34 of 1992/93 Biennial Report)

Data from previous epidemiological studies that found possible increases of lung, stomach and prostate cancers among lead smelters and workers in battery manufacture are being assembled for a common, thorough analysis. Data have already been obtained from cohorts in Italy, the UK and the USA.

2.2.1.7 *Cancer risk in the wood and leather industries* (pp. 34–35 of 1992/93 Biennial Report)

Data from previous epidemiological studies in China, Denmark, Finland, France, Germany, Italy, The Netherlands, Sweden, the UK and the USA have been analysed to allow a more specific evaluation of the cancer risk within the wood and leather industries in terms of chemical exposure or department/job of employment. A systematic assessment of exposure information has been conducted, including data that were not used or presented in published reports. Results indicate an increased risk of nasal adenocarcinoma among subjects exposed to wood dust, while no increased risk was found for nasal carcinoma. Data available were not sufficient to allow for specific analyses according to type of wood.

2.2.1.8 Cancer risk due to asphalt vapour (p. 35 of 1992/93 Biennial Report)

Vapour condensates from coal tar and bitumen were tested for DNA adduct formation in rats by ^{32}P-postlabelling. The patterns of adduct formed *in vitro*, using rat liver microsomes, were different with coal tar condensates and with bitumen condensates, probably because coal tar contains more polycyclic aromatic hydrocarbons and bitumen more polycyclic heterocyclic (sulfur, nitrogen) hydrocarbons. Initial results from skin painting with the vapour condensates *in vivo* indicate that at least as many adducts are found in the lung as in the skin of the treated animals, and that adduct levels in lymphocytes may reflect skin exposure to coal tar and bitumen vapours.

A pilot study in 15 European countries has evaluated the feasibility of a cohort study among workers involved in asphalt mixing and road paving. The study is feasible in Denmark, Finland, France, Germany, the Netherlands, Norway and Sweden. The protocol of the full study is now being finalized.

2.2.1.9 *Cancer risk among workers exposed to mercury* (p. 36 of 1992/93 Biennial Report)

A protocol has been established for an historical cohort study in the four mercury mines where the majority of European mercury was produced: Monte Amiata (Italy), Idrija (Slovenia), Almaden (Spain) and Nikitovka (Ukraine). Cohorts have been assembled and follow-up for mortality and cancer incidence will be completed in 1995.

2.2.1.10 *Chronic low-dose exposures to ionizing radiation* (pp. 37–39 of 1992/93 Biennial Report)

Studies of nuclear industry workers

The three-country study of cancer risk among nuclear industry workers has led to the most precise direct estimates to date of the risk of cancer associated with generally low-dose protracted exposure to ionizing radiation (IARC Study Group, 1994). A significant dose-related increase in leukaemia risk was found. The excess relative risk for death from leukaemia (excluding chronic lymphocytic leukaemia) was 2.2 per sievert. This estimate is slightly lower than the linear estimate derived from Japanese atomic bomb survivors' data. These results provide little evidence that the estimates that form the basis of current radiation protection recommendations are appreciably in error.

The international collaborative study of nuclear industry workers has been extended to include Hungary, the Slovak Republic and the USA. In the former two countries, a feasibility study is being evaluated. Data collection is proceeding in all other countries. A socio-economic status indicator is being used to adjust for possible confounding effects of life-style factors on the relationship between low-dose radiation and cancer risk. Cross-sectional surveys of smoking habits are being planned in most of the participating countries. A detailed protocol has been developed for the study of biases and random errors in the radiation dose estimates. The main aims of this study are: (1) to identify workers with substantial doses from internal contamination with radionuclides for which dose estimates may be inaccurate; and (2) to quantify the systematic and random errors in dose estimates for workers whose dose is predominantly from higher-energy γ-rays.

Epidemiological studies of the health consequences of the Chernobyl accident

In order to evaluate the feasibility of long-term epidemiological studies of cancer risk among emergency accident workers, three pilot studies were set up in Belarus and the Russian Federation within the framework of an experimental collaboration project of the European Commission.

The first study, a test of follow-up mechanisms, has been completed. A sample of 500 civilian emergency accident workers having worked in the 30 km zone around the Chernobyl reactor between May 1986 and December 1987, was chosen at random. Vital status of these subjects at the end of study date (1 October 1993 in Belarus and 31 December 1992 in Russia), and the date and cause of death for deceased individuals, were ascertained. Follow-up was

virtually complete (99%) in both countries. In Belarus, 80% of the population could be traced using only two different sources of information and in the Russian Federation, it was possible to follow up 85% of the population using three sources of information. A long-term study using this approach could therefore be envisaged.

The second study is a pilot case-control study of leukaemia among emergency accident workers. Most of the cases have now been identified; approaches for selecting controls are being evaluated; the questionnaire, which has been adapted from the questionnaire of the Estonian study of emergency accident workers, is being tested. Data collection should be completed in October and the results should be available by the end of 1994.

The third study is a validation and calibration of biological dosimetric techniques. The aim is to assess the feasibility of distinguishing between subjects having received low and high radiation doses, by analysing stable chromosomal aberrations in blood cells collected several years after the accident. A stratified random sample of 60 emergency accident workers (20 each with total registered dose between 0 and 100 mSv, 100 and 200 mSv and greater than 200 mSv) residing in the Obninsk area who worked in the 30 km zone around the Chernobyl reactor between May 1986 and December 1987 was chosen for this study, the first results of which are expected by the end of 1994.

A small-scale survey of childhood thyroid cancer cases was carried out in collaboration with the Institute of Radiation Medicine in Minsk in order to obtain additional information on the recently reported increase in this disease in Belarus (Kazakov *et al.*, 1992). In 80% of the 50 children (64% girls), the thyroid tumour was diagnosed as a result of screening (either the annual medical examination at school or a formal screening programme). Four of the mothers and eight of the fathers had participated in the clean-up of the Chernobyl accident. Approximately half of the children had slept with open windows and the majority (64 %) had spent 8 hours a day or more out of doors in the days following the Chernobyl accident. The majority of cases also consumed locally produced milk and fresh leafy vegetables around the time of the accident. In 36% of the cases, the child usually drank water from an open well. Only eight children had received iodine prophylaxis in early May 1986. All 50 children were below the age of 10 at the moment of the accident: 3 were not yet born and 54% were below the age of 5.

2.2.2 Occupational cancer in industrially developing countries (pp. 39–41 of 1992/93 Biennial Report)

A number of specific studies on occupational cancer risks have been started in selected areas of developing countries: (i) proportionate mortality and cohort studies among steel

workers in Vale do Aco, Brazil, with special emphasis on lung cancer and leukaemias; (ii) a case–control study on bladder cancer and tobacco smoking, *Schistosoma* infestation and occupational exposures in Alexandria, Egypt (Bedwani *et al.*, 1993); (iii) a multi-site case–control study on occupational risk factors in Bombay, Madras and Trivandrum, India; (iv) a multi-site case–control study on occupational risk factors in Montevideo, Uruguay; (v) a cohort study on rubber workers in Shanghai, China. A book on occupational cancer in developing countries is in press (Pearce *et al.*, 1994b). The feasibility of additional projects on lung cancer in Argentina and Brazil, and on textile workers from several developing countries, is under study.

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2.3 Diet, nutrition and cancer

2.3.1 European Prospective Investigation into Cancer and Nutrition (EPIC) (pp. 44–48 of 1992/93 Biennial Report)

The EPIC project is a multi-centre prospective cohort study designed to investigate the relation between diet, nutritional status, various lifestyle and environmental factors and the incidence of different forms of cancer. The main features of the projects are:

- 350 000 middle-aged men and women are being recruited in 17 regional centres located in seven European countries.
- Detailed information is obtained on: usual diet, physical activity, tobacco smoking, alcohol consumption, occupation and socioeconomic status, reproductive history, contraception and hormone replacement therapy, previous illnesses and current drug use.
- Weight, height, waist and hip circumferences and sitting height are measured using standard procedures.
- Blood samples are collected, separated into 28 small aliquots of serum, white blood cells and red blood cells, and stored in liquid nitrogen (−196°C) for future analyses.

Recruitment of subjects and collection of data and biological samples have progressed in all centres (see Table 1).

In Spain, more than 50% of the planned cohort has been recruited with complete data and biological samples. In Italy, the UK and the Netherlands, about 25–30% of the total cohort has been recruited by September 1994. In Germany and Greece, field work started in spring 1994. In France, 77 000 subjects have returned two mailed questionnaires on non-dietary variables, and about 50 000 of them have also returned the dietary questionnaire. Collection of blood samples is beginning.

An important development has been the establishment of a formal and close collaboration with a similar prospective study which was started in Denmark in 1994.

An innovative component of the EPIC project has been the development of methodology for the calibration of dietary measurements, standardization of dietary

assessment methods (see Section 2.3.2) and the setting up of a bank of biological samples stored in liquid nitrogen at IARC. During 1993–94, samples of plasma, serum, white blood cells and erythrocytes from about 45 000 subjects were received and stored in liquid nitrogen. About 630 000 0.5-ml aliquots (14 aliquots per subject) are now stored at IARC. Looking forward to the follow-up period, a new conceptual and statistical approach has been developed to improve the efficiency of the use of the samples once cancer cases are identified in the EPIC cohort, in order to test as many biological hypotheses as possible, while keeping to a minimum the wastage of samples on testing hypotheses which are likely to be rejected. This new approach is based on sequential laboratory determinations and sequential statistical testing of the results (Kaaks *et al.*, 1994a; van der Tweel *et al.*, 1993).

2.3.2 Methodology for calibration and standardization of dietary measurements in nutritional epidemiology (pp. 48–49 of 1992/93 Biennial Report)

Work has progressed along three parallel and complementary lines:

(*a*) *Statistical methodology*: New *statistical concepts* regarding calibration and validation of dietary intake measurements obtained in prospective cohort studies (Kaaks *et al.*, 1994b,c,d; Plummer *et al.*, 1994) have been developed for use in the EPIC study. Dietary intake is being measured by means of questionnaires and food records adapted to local culture and dietary patterns. To correct for systematic differences in terms of over- or underestimation of dietary intake, a second measurement of diet will be made in a subsample of 10% of the cohort (3000–4000 subjects per country) with a strictly standardized 24-hour diet recall method.

(*b*) *Development of a standardized and computerized 24-hour diet recall method*: A 24-hour diet recall method has been developed based on new criteria for standardizing of data collection between countries. In parallel, a computer program (EPIC-SOFT) is being produced, with country-specific versions for each of the participating countries. A complex system has been devised to standardize and compare the definitions of foods, recipes, ingredients, cooking methods and the many other variables which determine the composition and the amount of foods eaten in different countries. A food picture book containing six standard portion sizes of each of 90 foods has been produced and will be used for the quantification of food portions in the 24-hour diet recall (van Kappel *et al.*, 1994).

(c) *Food composition tables for nutritional epidemiology:* The work of setting up the large food composition databases required for epidemiological studies on diet and cancer has progressed. Food composition tables for French and Italian foods are in press (Slimani *et al.*, 1994a,b). Similar tables for Spanish foods are now being finalized.

2.3.3 The New York University Women's Health Study (p. 49 of 1992/93 Biennial Report)

This prospective study, initiated in 1985, includes 15 500 women living in the New York metropolitan area; blood samples were collected in 1985–86 from all women in the cohort. Follow-up for cancers of the breast, colorectum, endometrium and ovary will continue until 1998. Results for the first 180 incident cases and 900 matched controls show that high consumption of meat and saturated fat doubled the incidence of breast cancer (Toniolo *et al.*, 1994).

2.3.4 Malmö Prospective Study on Diet and Cancer (pp. 49–50 of 1992/93 Biennial Report)

Collection of data and blood samples from middle-aged men and women living in the Malmö area has continued, the number of subjects having now reached about 18 000.

2.3.5 Case–control studies on diet and stomach cancer in different populations of Europe (pp. 50–51 of 1992/93 Biennial Report)

Various hypotheses on the links between dietary habits and occurrence of gastric cancer have been investigated in three case–control studies conducted in Belgium, Spain and France. The three studies provided very concordant results regarding the inverse association between risk of gastric cancer and consumption of fruit, vegetables and some related micronutrients (González *et al.*, 1994a). The Spanish study also examined the relation between gastric cancer risk and consumption of tobacco and alcohol, and found that only adenocarcinoma of the cardia was strongly associated with both alcohol and tobacco intake.

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Table 1. Progress in data collection in the EPIC project (September 1994)

	Spain	Italy	UK	Netherlands	France	Greece	Germany	TOTAL
Questionnaires:								
Starting date	Oct -Nov. 1992	Jan.-Sept. 1993	Jan. 1993	Jan. 1993: Morgen June 1993: Prospect	May 1992	Feb. 1994	June-Sept. 1994	
No. collected	24 000	11 800	10 500	11 600	77 000[a] 50 000[b]	1500	500	136 900
Daily average	70	55	46	40	N.A.	20[c]	70[d]	301
Blood collection:								
Starting date	Oct.-Nov. 1992	Jan.-Sept. 1993	Jan. 1993	May 1993	Sept. 1994	Feb. 1994		
No. of samples collected	22 000	12 900	8500	9600	-	2500	500	56 000
Daily average	70	60	46 .	40	-	20[c]	70[d]	306
Forecast for Dec. 1996:								
Questionnaires	} 40 000	} 40 000	} 40 000	} 31 000	80 000	} 40 000	} 35 000	306 000
Blood samples					35 000			261 000
Expected final size of cohort	40 000	50 000	40 000	40 000	80 000	40 000 35 000	55 000	345 000[e] 300 000[f]
End of data collection	Dec. 1995	June 1997	Dec. 1996	Dec. 1997	June 1996	Dec. 1996	Dec. 1998	

[a] Two questionnaires on lifestyle only

[b] Questionnaire on diet + lifestyle

[c] Expected to increase to 40 per day by the end of 1994 x 7 days per week

[d] Expected rate after short initial period at lower rate

[e] Questionnaire data only.

[f] Questionnaire data plus blood samples

2.4 Tobacco and cancer

2.4.1 Case–control study of lung cancer and environmental tobacco smoke in non-smokers (p. 57 of 1992/93 Biennial Report)

A multicentric case–control study on lung cancer among non-smokers is under way in 11 European centres. Data collection was completed in 1993, and the analysis is in progress. Two additional studies are being conducted in some of the participating centres: a project on genetic polymorphism for cytochrome P450 enzymes and DNA repair activity to investigate susceptibility to exposure to low levels of carcinogens, and a project on active smokers to investigate the interaction between active smoking, occupation and other risk factors in the etiology of lung cancer.

2.4.2 Smoking in France (p. 58 of 1992/93 Biennial Report)

Studies are conducted regularly at both local and national levels to evaluate trends in smoking among the French adult and adolescent populations. A decrease in smoking prevalence is seen among young people (Sasco, 1993c). Peer behaviour is a strong determinant of smoking and substance abuse among this age group (Sasco *et al.*, 1993). In the French adult population, the proportion of current smokers is decreasing among men (Sasco, 1994b; Sasco *et al.*, 1994a) but not among women, more markedly in the most affluent social classes (Sasco *et al.*, 1994b), as in other southern European countries. In France, no decrease has yet been seen in lung cancer mortality (Sasco, 1993a).

2.4.3 Evaluation of the efficacy of various anti-smoking strategies (p. 58 of 1992/93 Biennial Report)

The impact is being evaluated of legislative and educative strategies on smoking habits among various groups such as children, young people, the working population and health-related groups. Legislative action appears to have the greatest impact and is most obvious for the effect on passive smoking (Sasco, 1994a).

2.4.4 Tobacco use in Africa (pp. 58–59 of 1992/93 Biennial Report)

Preliminary estimates have been produced of lung cancer incidence attributable to tobacco use worldwide (Parkin & Sasco, 1993; Sasco, 1994c) and for Africa (Sasco, 1994d). A

Bibliography

multicentric case–control study has been proposed in order to obtain more precise using a protocol similar to one already used in China (Liu *et al.*, 1993).

2.4.5 Cohort study of tobacco use and mortality in Bombay, India (p. 59 of [illegible] Biennial Report)

The project aims to study the risks of tobacco use for cancer and cardiovascular and respiratory diseases in India, with mortality as the end point. Enrolment into the cohort started in 1991, and by June 1994, almost 100 000 individuals aged at least 35 years had been interviewed. Enrolment is continuing, now restricted to men aged 45 years and over. Data collection from the municipal death registration system is in progress and methodological issues are being addressed (Gupta *et al.*, 1994).

2.4.6 Biological and chemical characterization of substances in human urine strongly inhibiting bacterial mutagenicity of aromatic and heterocyclic amines (p. 61 of 1992/93 Biennial Report)

Tobacco smoking causes a major fraction of urinary bladder cancer in humans. Various studies have implicated primary aromatic and possibly heterocyclic amines, which are present in tobacco smoke, as bladder carcinogens (Bartsch *et al.*, 1993). Human urine contains substances that strongly inhibit the bacterial mutagenicity of such amines (Malaveille *et al.*, 1992). The bacterial anti-mutagenicity of urine from 10 smokers has been shown to be inversely related to the level of DNA adducts in exfoliated urinary bladder cells (isolated from urine) arising from exposure to aromatic and heterocyclic amines. Experimental evidence indicates that this activity is due to dietary polyphenols.

2.4.7 Pulmonary carcinogen–DNA adducts, cytochrome P450 enzymes, smoking and occupational exposure in lung cancer patients in Finland (pp. 59–60 of 1992/93 Biennial Report)

The analysis of lung samples for DNA adducts by ^{32}P-postlabelling and for various enzymes has continued on new samples obtained from the Institute of Occupational Health (Helsinki, Finland). The data are in the process of analysis.

2.4 IARC staff publications

Bartsch, H., Malaveille, C., Friesen, M., Kadlubar, F.F. & Vineis, P. (1993) Black (air-cured) and blond (flue-cured) tobacco cancer risk. IV: Molecular dosimetry studies implicate aromatic amines as bladder carcinogens. *Eur. J. Cancer*, **29A**, 1199–1207

Gupta, P.C., Sankaranarayanan, R. & Vainio, H. (1994) Smokeless tobacco use and oral cancer. *Oral Oncol. Eur. J. Cancer*, **30B** (in press)

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2.5 Urinary tract cancer in relation to Balkan endemic nephropathy and exposure to ochratoxin A and other mycotoxins

2.5.1 Epidemiology of urinary tract tumours in Bulgaria (p. 64 of 1992/93 Biennial Report)

Data collected in Bulgaria during 1992–94 are being analysed in collaboration with Dr N. Day (Cambridge, U.K.). Plans have been made for a retrospective study using data on ochratoxin A exposure and the new epidemiological data.

Recent data from Tunisia have demonstrated that all patients with nephropathy of unknown origin had high ochratoxin A blood levels (25–100 ng/ml) and more renal tumours have been observed in dialysis patients (Bach & Bartsch, 1993).

2.5.2 Mechanism of action of ochratoxin A (p. 65 of 1992/93 Biennial Report)

In the two-year rat experiment performed in collaboration with Professor U. Mohr (Hannover), ochratoxin A induced kidney tumours in male DA and Lewis rats, and to a lesser extent in female Lewis rats but not in female DA rats. This is consistent with the previous hypothesis that slow metabolizers of debrisoquine may be less susceptible to ochratoxin A carcinogenicity than fast metabolizers (Castegnaro *et al.*, 1989). Administration of 2-mercaptoethanesulfonic acid sodium salt (Mesna) did not reduce the incidence of tumours in either strain. A higher level of DNA adducts was detected by ^{32}P-postlabelling in kidneys of male than in those of female rats following ochratoxin A exposure. The analysis of adducts in the other organs is in progress. Alterations of the kidney function are being examined by analysis of urinary γ-glutamyltranspeptidase, leucine aminopeptidase, lactate dehydrogenase from the animals during the two-year experiment.

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2.6 Role of viruses in the etiology of human cancer

2.6.1 Collection of biological material related to EBV and lymphomas (p. 67 of 1992/93 Biennial Report)

The biological samples have been used by various external investigators, leading to several publications. Serum interleukin-10 was found to be a prognostic factor in malignant non-Hodgkin lymphoma (Blay *et al.*, 1993).

Further studies have been performed on the importance of 6q deletions in Burkitt's lymphoma cell lines (Parsa *et al.*, 1994), following the initial collaboration with Dr R. Dalla-Favera.

The Ewing sarcoma cell lines established at IARC were used to better define the molecular rearrangement involving chromosomes 11 and 22 (Zucman *et al.*, 1993). It was shown that the subgroup of small-round-cell tumours identified as belonging to the Ewing family of tumours can be defined according to a specific molecular genetic lesion that is detectable by a rapid, reliable, and efficient method. This approach can be applied to small specimens obtained by fine-needle biopsies (Delattre *et al.*, 1994).

2.6.2 Studies on lymphomas occurring in AIDS patients (p. 67 of 1992/93 Biennial Report)

The studies on EBV-associated lymphoma have been phased out. A report has been made on the analysis of 36 cases of AIDS lymphomas (Raphael *et al.*, 1994).

2.6.3 Case–control study of nasopharyngeal carcinoma in relation to infection with Epstein–Barr virus and exposure to other agents in south-east Asia (p. 68 of 1992/93 Biennial Report)

Pilot studies have been conducted in Thailand and Viet Nam to develop appropriate questionnaires on usual dietary habits in order to investigate possible risk factors in the local diets.

24-hour recall interviews have been conducted in Khon Kaen (Thailand) and Hanoi (Viet Nam); the data from the Thai centre have been analysed. A first version of the questionnaire has been field-tested and the final version is now available. The pilot study in Hanoi has been completed and the data are being analysed.

2.6.4 Case–control studies of Kaposi's sarcoma, non-Hodgkin lymphoma and carcinoma of the cervix in Africa in relation to infection with HIV (p. 68 of 1992/93 Biennial Report)

The initial project, in Rwanda, was proceeding well until the civil unrest of April 1994. Results up to September 1993 are available and some preliminary analyses have been undertaken.

A larger-scale project began in February 1994 in Kampala, Uganda, supervised by a senior scientist on secondment to IARC. The initial emphasis is upon risk factors for Kaposi's

sarcoma in adults and in children, including testing a hypothesis of an association with soil type (Ziegler, 1993).

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2.7 Endogenously formed carcinogens in human cancer etiology

2.7.1 Chronic infection/inflammation and human cancer (p. 70 of 1992/93 Biennial Report)

Chronic infection and inflammation are recognized risk factors for a variety of human cancers. Active oxygen and nitrogen species, such as hydroxy radical and nitric oxide (NO) radical, generated in inflamed tissues, can cause injury to target cells and also damage DNA, which could contribute to tumour development (Ohshima & Bartsch, 1994; Calmels & Ohshima, 1994).

Since an inducible form of NO synthase produces large quantities of NO which may cause cytotoxic and mutagenic effects, the presence and induction of NO synthase are being studied in normal, precancerous and tumour parts of human and animal specimens. Increased activity and immunoreactivity of an inducible NO synthase were detected in human liver specimens from cirrhosis patients (Ohshima *et al.*, 1994a), in the liver of hamsters infected with *Opisthorchis viverrini* (Ohshima *et al.*, 1994b) and in the liver of rats with massive

hepatic cell necrosis (Bandaletova *et al.*, 1993) . We have cloned complementary DNA of an induci. e NO synthase from rat liver, in collaboration with Dr H. Esumi, Tokyo, Japan (Adachi *et al.*, 1993).

In order to investigate the relationship between key enzymes involved in oxidative stress of the gastric mucosa and the intensity of *Helicobacter pylori* infection, we have carried out immunohistochemical analysis of inducible NO synthase and antioxidant defence enzymes (i.e., catalase (CAT) and Mn and Cu/Zn forms of superoxide dismutases (SOD)) in gastric biopsy samples collected from 94 patients with various precancerous or cancerous conditions (in collaboration with Dr B. Bancel, Professor L.M. Patricot, R. Lambert and B. Moulinier, Lyon, France). Increased expression of inducible NO synthase, CAT and Mn-SOD was more frequently observed in gastric biopsies infected with *H.* pylori than those without *H. pylori*.

Levels of nitrite, nitrate and *N*-nitroso compounds were significantly increased in the urine of rats with urinary tract infection through activation of inducible NO synthase and bacterial production of NO. Levels of oxidized proteins and expression of anti-oxidant enzymes and inducible NO synthase were higher in bladder tissues from infected rats. Experiments are being carried out to investigate the influence of NO and other DNA-damaging agents on the expression of the Mn-SOD.

In order to investigate the interrelationship between production of reactive species, host anti-oxidant defence and the resulting oxidant injury, methods to measure DNA damage are being explored (in collaboration with Drs J. Cadet, M. Polverelli and F. Berger, Grenoble, France). These methods are being used to study the effectiveness of anti-oxidant treatments and *Helicobacter pylori* eradication in intervention studies of stomach cancer (in collaboration with the Unit of Field and Intervention Studies and with M. Crespi, Rome, Italy and A.T.R. Axon, Leeds, UK).

2.7.2 DNA and tissue damage induced by endogenous free radicals

NO reacts rapidly with superoxide anion ($O_2^{\cdot}$) to form peroxynitrite ($ONOO^-$) which may itself be tissue-toxic and may decompose to the highly reactive and toxic hydroxy radical ($HO^{\cdot}$) and nitrogen dioxide ($NO_2^{\cdot}$). These radicals are implicated as the compounds responsible for tissue damage induced in infected and inflamed tissues. We have found that reaction of peroxynitrite with DNA leads to formation of DNA base-propenal derivatives. Similarly, its reaction with guanine resulted in the formation of oxidized or nitrated guanine derivatives, which are now being characterized.

Peroxynitrite also reacts with tyrosine residues in protein to form both nitrotyrosine and dityrosine. Anti-oxidants such as ascorbic acid and uric acid inhibited their formation.

These tyrosine modifications may serve as markers of peroxynitrite-mediated tissue damage and oxidative stress. Short-term and long-term animal experiments have been initiated to study the effects of peroxynitrite on tumour induction and promotion.

Cancer development and tumour promotion or progression could be impeded by reducing tissue damage induced by reactive oxygen/nitrogen species in inflamed tissues. We have studied the effect of various non-steroidal anti-inflammatory agents on the induction of NO synthase in macrophages by lipopolysaccharide and interferon-γ. Curcumin, a potent anti-tumour promoter, inhibited NO synthase induction at very low concentrations and we are studying its molecular mechanisms. We are also studying the effect of long-term feeding of NO synthase inhibitors on cholangiocarcinoma development in hamsters infected with *O. viverrini*.

In collaboration with other laboratories, the role of NO in tumour-induced immunosuppression (Lejeune *et al.*, 1994), pathogenesis of cerebral malaria (Asensio *et al.*, 1993) and formation of carcinogenic *N*-nitroso compounds in subjects infected with *O. viverrini* in north-east Thailand have been studied. It appears that the infected subjects excrete higher levels of *N*-nitrosodimethylamine than non-infected subjects, after consumption of beer (ethanol) which inhibits metabolism of the nitrosamine. Statistical analysis is under way.

2.7.3 Effect of gastric/urinary bacteria infection on formation of carcinogenic *N*-nitroso compounds and their precursors (p. 74 of 1992/1993 Biennial Report)

Decreased gastric acidity caused by antacid drugs permits proliferation of bacteria, leading to *in situ* formation of nitrite and carcinogenic *N*-nitroso compounds. In collaboration with Drs E. Verdu, F. Viani, D. Armstrong, R. Fraser, H.H. Siegrist, A.L. Blum and M. Fried (Lausanne, Switzerland) we have measured levels of nitrate, nitrite, total *N*-nitroso compounds in gastric juice samples from volunteers and patients treated with omeprazole and correlated with the degree of bacterial infection. Although bacterial counts increased significantly after omeprazole treatment, there was no increase in the concentrations of nitrite, nitrate and total *N*-nitroso compounds (Verdu *et al.*, 1994).

Patients with urinary diversion are at elevated risk for cancer in the ureterocolic anastomosis after ureterosigmoidostomy, in reconstructed tissue after cystoplasties performed for contracted bladder or in colonic conduits. They commonly suffer from urinary tract bacterial infection. In collaboration with Drs A.L. Poulsen, K. Steven and J. Carstensen, Herley, Denmark, we are measuring the extent of bacterial infection and the excreted amounts of *N*-nitroso compounds and their precursors in the urine of patients with urinary diversion with an ileal Kock reservoir and in control patients. The results are being analysed statistically.

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2.8 DNA damage and its relationship to cancer

2.8.1 Detection of carcinogen-specific mutations in oncogenes and tumour-suppressor genes before tumour appearance (pp. 78–82 of 1992/93 Biennial Report)

UV-specific p53 gene mutations are detected in biopsies of normal skin only from sun-exposed sites but not non-exposed sites (Nakazawa *et al.*, 1994). On the basis of this finding we are conducting case-control studies of sun exposure and skin cancers. Samples of normal skin from cases and controls from Geraldton, Australia (Kricker *et al.*, 1990, 1993) are being analysed for the presence of UV-specific CC to TT mutations in the p53 gene. A higher prevalence of the p53 gene mutations in cases than in controls will imply that p53 gene mutation measurement may be useful for prediction of cancer risk due to UV exposure. On the

other hand, if the prevalence of mutation is the same in case and control groups, it will imply that this method is useful as a cumulative measure of exposure to sunlight.

We are comparing the frequency of specific mutations in the p53 gene that give cells a growth advantage with the frequency of CC to TT mutation at codon 91, that does not lead to any change of amino acid and is therefore silent in terms of cellular growth. These measurements in normal skin fibroblasts and keratinocytes and those from xeroderma pigmentosum patients will indicate whether there are different degrees of accumulation of these types of mutation. Measurements of the frequency of the silent mutation may provide the best indication of cumulative UV exposure.

2.8.2 **Microsatellite DNA changes in human and carcinogen-induced tumours** (p. 97 of 1992/93 Biennial Report)

Alterations in microsatellite DNA sequences often occur in human tumours at multiple loci (Aaltonen *et al.*, 1993; Thibodeau *et al.*, 1993). Such genetic alterations occur in familial as well as sporadic cancers, and are considered to be the result of induction of a mutator phenotype (Jiricny, 1994). We are using microsatellite DNA changes as a model to study the role of genetic instability in carcinogenesis.

To extend our work on the presence of microsatellite DNA changes in primary human stomach cancer (Mironov *et al.*, 1994), we have examined human oesophageal cancers and again found microsatellite DNA changes. Two out of 18 human squamous cell carcinomas of the oesophagus contained a change of (CA)n repeats. In addition, loss of heterozygosity (LOH) was observed in three samples. Among 32 oesophageal papillomas induced by *N*-nitrosomethylbenzylamine in the rat, we found five that contained (CA)n repeat changes and two with LOH. Both (CA)n repeat length alteration and LOH were clustered at certain loci in the human tumour samples as well as in the chemically-induced rat oesophageal tumours.

Among many microsatellite alterations found at various loci in a given tumour, one or more of these loci are believed to be involved in carcinogenesis, but no definite target genes have been identified. As a possible target, we have analysed the locus of a DNA repair gene, ERCC1 gene; this gene is located at chromosome 9q13.2-q13.3 and its ninth intron contains a $(CA)_7$ repeat; another repeat $(CA)_{13}AAAAG(CA)_5$ is located at an upstream position. Among 22 gastric cancer samples, we found two that contained an altered (CA)n repeat at the ninth intron and another two contained changes at upstream microsatellite DNA sequences. These results provide the first example of a possible target which may be affected by genomic instability through microsatellite changes.

While we considered that BALB/c 3T3 cells are genomically unstable due to the fact that they are readily transformable by various carcinogens, our results suggest that no microsatellite DNA changes occur after transformation of these cells. In addition, we have tested mouse skin tumours induced by a two-stage protocol of carcinogenesis, and none of them contained microsatellite DNA changes. These results suggest that microsatellite DNA changes may be important for carcinogenesis of some, but not all, types of cells and/or tissues.

2.8.3 *p53* gene mutations in liver cancers induced by vinyl chloride in rats (pp. 83–84 of 1992/93 Biennial Report)

Fifteen angiosarcomas of the liver and eight hepatocellular carcinomas from Sprague-Dawley rats exposed to vinyl chloride are being analysed for *p53* mutations, using PCR amplification and sequencing of exons 5 to 8 of the gene. To date, *p53* mutations have been found in exon 6 in two samples: one is an AT→GC transition at the second nucleotide of codon 203, the other is a GC→AT transition at the second nucleotide of codon 246. An AT→TA tranversion at the second nucleotide of codon 283 (exon 7) has been observed in one hepatocellular carcinoma induced by vinyl chloride. Further samples of rat liver angiosarcomas provided by Professor C. Maltoni (Bologna, Italy) are under investigation.

2.8.4 Mutations of *p53* in oral cancer (p. 116 of 1992/93 Biennial Report)

We examined 20 oral squamous cell carcinomas from Baltimore, USA, and 30 from Papua New Guinea (PNG), for mutations in exons 5–9 of the *p53* gene. Mutations were found in three of the tumours from PNG (10%), whereas nine mutations were detected among those from Baltimore (45%). In agreement with the low number of PNG cancers with mutations, only 17% of the cases from PNG were positive by immunostaining. The presence of human papillomavirus DNA in PNG cases was examined with a polymerase chain reaction-based procedure, and viral sequences (human papillomavirus strains 11/16) were detected in two tumours. Human papillomavirus-triggered degradation of the tumour-suppressor protein is thus unlikely to be a typical pathway to *p53* dysfunction in tumours from PNG.

2.8.5 *p53* mutations in cancers attributable to occupational exposure (p. 84 of 1992/93 Biennial Report)

2.8.5.1 *p53 mutations at A:T base pairs in angiosarcomas of vinyl chloride-exposed factory workers*

Occupational exposure to vinyl chloride causes liver angiosarcomas (ASL) and also increases the risk for several other cancers. We examined tumours from five vinyl chloride-exposed patients, four with ASL and one with hepatocellular carcinoma, for evidence of MDM2 proto-oncogene amplification or *p53* mutation in exons 5–8. Amplification of MDM2 was not found, but in two angiosarcomas an A:T to T:A missense mutation was detected.

2.8.5.2 *Genetic alterations in bladder cancers attributable to exposure to aromatic amines*

We have conducted an analysis of *p53* and H-*ras* gene mutations and *p53* protein accumulation in 11 tumour samples from German workers in the dye industry exposed to benzidine and, in some cases, to 2-naphthylamine and 4-aminobiphenyl. Since most of the workers were also smokers, we analysed in parallel *p53* and H-*ras* mutations in 11 transitional cell carcinomas of the bladder from Turin, Italy, from smokers and non-smokers who had no record of occupational exposure. Preliminary data show that *p53* mutations are present in one third of the cases, while the frequency of *ras* mutation is much lower.

2.8.6 Second malignancies following chemotherapy

2.8.6.1 *Epidemiological studies of second malignancies* (pp. 85–86 of 1992/93 Biennial Report)

Two pilot studies on statistical variability of markers of DNA damage among testicular cancer patients treated with cisplatin and among Hodgkin's disease patients treated with alkylating agents have been completed. A multicentric prospective study has been initiated in about 10 centres; blood samples are being collected from Hodgkin's disease patients undergoing chemotherapy. These patients are then followed up for occurrence of second cancer and clinical response. Ancillary projects are planned on kinetics of DNA adducts, risk of non-Hodgkin lymphoma following chemotherapy for Hodgkin's disease, and etiological factors for Hodgkin's disease.

2.8.6.2 *Markers of DNA damage and risk of second malignancy in Hodgkin's disease patients* (p. 86 of 1992/93 Biennial Report)

An HPLC-electrochemical detection method to measure 7-methylguanine (7-meG) has been applied to peripheral blood cell DNA from patients treated with procarbazine for Hodgkin's disease. Of 19 such patients, sampled at the end of the first cycle of chemotherapy, all but one had detectable levels of 7-MeG (>1 pmol/μmol guanine) and there was an approximately five-fold interindividual variation. Only two patients had detectable levels prior to any chemotherapy and these levels were only slightly above the detection limit.

2.8.7 Detection of DNA methylation adducts following exposure to environmental methylating agents (p. 87 of 1992/93 Biennial Report)

A new technique has been established using ^{32}P-postlabelling chromatography and immuno-purification and this offers increased sensitivity for measurement of *O*-alkylated bases, allowing detection of 1 fmol O^6-methylguanine and O^4-methylthymidine.

7-Methylguanine (7-MeG) levels in peripheral blood cells (PBC) and internal organs in rats exposed to different methylating agents (nitrosodimethylamine, 1,2-dimethylhydrazine, 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone and *N*-nitrosomethylbenzylamine) have been compared in order to validate the use of PBC as surrogate cells for internal organs in epidemiological studies. All of these methylating agents led to formation of 7-MeG in PBC but the ratio of the 7-MeG levels in target organ and PBC varied by two orders of magnitude between carcinogens. In contrast, the ratio between the adduct level in PBC and liver was relatively constant for all carcinogens. These observations were the same for different routes of carcinogen administration. The results suggest that 7-MeG levels in PBC following exposure to methylating agents could be predictive of those in the liver (Bianchini & Wild, 1994a,b,c).

2.8.8 Prediction of carcinogenic potency of genotoxic chemicals (pp. 87–89 of 1992/93 Biennial Report)

With the aim of characterizing different classes of genotoxic carcinogens and establishing quantitative structure/activity relationships, the carcinogenic potency of various chemicals in rodents is being compared with their DNA adduct patterns and their genotoxic activity profiles and mutation spectra in *Drosophila*. This approach has allowed compounds yielding DNA etheno-adducts (vinyl halides, urethane) to be characterized as a new class of carcinogens. These chemicals show a relative clastogenic efficiency (ratio of chromosome

aberrations to forward mutations) similar to that of cross-linking agents. However, in contrast to cross-linking agents, they show a hypermutability effect when the nucleotide excision repair pathway is not functioning. Thus, they form a group of genotoxic agents which, on the basis of their activity profiles, are clearly separable from both monofunctional and cross-linking alkylating agents.

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2.9 Cancer of the oesophagus

2.9.1 Case–control studies in high-risk populations of Latin America (p. 91 of 1992/93 Biennial Report)

The pooled analysis of five case-control studies involving 830 cases and 1779 controls showed that alcohol and tobacco are the main risk factors. Heavy drinkers and smokers have a risk 76 times higher than non-drinkers and non-smokers. Mate drinking had a moderate independent effect, especially among females and non-drinkers of alcohol.

2.9.2 The induction of cytochrome P450 2E1 by various alcoholic drinks

Different alcoholic drinks (farm calvados, commercial calvados, red wine, cider, whisky and beer) were administered to rats in drinking water for three days and microsomes were prepared from oesophagus and liver in order to measure cytochrome P450 levels by Western blot and enzyme activity. For all drinks except calvados, levels of hepatic induction of CYP 2E1 were similar to those in the control group exposed to an equivalent amount of ethanol. The calvados appeared to induce CYP 2E1 more than an equivalent amount of ethanol. Other isoenzyme levels and activities are being studied and the analyses will be extended to include the oesophageal P450 levels.

2.9.3 Genetic alterations in oesophageal cancer

The detection of *p*53 mutations in samples originating from high-risk areas in China has continued. In addition, methods to detect mutation of other genes (namely *p*16) involved in cell cycle control have been developed.

2.9 IARC staff publications

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2.10 Cancer of the stomach

2.10.1 Case-control study in Tachira, Venezuela (p. 96 of 1992/93 Biennial Report)

A total of 162 cases and 248 controls have been interviewed and biological specimens collected. A preliminary analysis of the first 119 cases and their controls suggested an increased risk associated with high consumption of corn and low intake of fruit and vegetables, and no association with *H. pylori*. In view of these interesting initial results, the Scientific Council recommended continuing recruitment up to at least 200 cases and 400 controls.

2.10 IARC staff publications

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2.11 Cancer of the liver

2.11.1 Cohort studies of hepatocellular carcinoma in HBsAg carriers in Thailand and Catalonia (p. 99 of 1992/93 Biennial Report)

Follow-up of the cohort of 1972 HBsAg carriers from Bangkok continues. Efforts are being concentrated on tracing 376 subjects who did not return for the second follow-up and about 200 subjects who have liver abnormalities. Needle aspiration biopsies are being performed in the latter to establish a clear diagnosis.

The follow-up of the 2515 HBsAg carriers from Catalonia will be completed by Dr F.X. Bosch in Barcelona.

2.11.2 Epidemiology of cholangiocarcinoma in Thailand (pp. 99–100 of 1992/93 Biennial Report)

The recruitment of the cohort continues. The number of subjects fully enrolled (blood, urine, faeces and interview data collected) by June 1994 was 4224. A new dietary questionnaire has been designed based on the results of a cross-sectional survey. Pilot studies on markers of activity of some cytochrome P450 isoenzymes in relation to infection with *Opisthorchis viverrini* have been started.

2.11.3 Human exposure to aflatoxin, metabolism, hepatitis infection and liver enzymes (p. 101 of 1992/93 Biennial Report)

The hypothesis that liver injury induced by hepatitis B virus, or other agents such as hepatitis C virus, can alter hepatic carcinogen metabolism is being examined in a large study of chronic carriers and non-carriers in The Gambia. Over 350 subjects have been tested for aflatoxin–albumin adducts, glutathione *S*-transferase M1 genotype, markers of hepatitis B and C infection and serum transaminases. The same subjects are now being phenotyped for cytochrome P450 3A4 activity and the prevalence of serum antibodies to p53 protein is being assessed.

In a second study, in Guinea, the prevalence of exposure to aflatoxin and to hepatitis B and C viruses was determined in 75 subjects. Over 90% were positive for aflatoxin–albumin adducts in sera and 15% were chronically infected with HBV. Eight subjects were positive for HCV antibodies. A more comprehensive survey is in progress to establish prevalence of exposure in four regions of the country in two different seasons as a basis for developing and targeting intervention measures to reduce aflatoxin exposure.

2.11.4 **Experimental studies of aflatoxin metabolism and carcinogenicity** (pp. 102–103 of 1992/93 Biennial Report)

2.11.4.1 *Hepatitis B transgenic mice*

Two strains of HBV transgenic mice exhibiting either chronic progressive liver injury due to accumulation of HBsAg in hepatocytes or an acute liver injury mediated by a cytotoxic T cell response against hepatocytes bearing HBV antigens have been examined for expression of a range of cytochrome P450s by immunohistochemistry, Western blot and enzyme activity. These studies show that levels of several P450s are increased during liver injury in these two models. The increase is particularly strong in the acute liver injury model and the mechanistic basis for this increase is being studied (Kirby *et al.*, 1994).

2.11.4.2 *Peking duck*

No mutations in codon 249 of the *p*53 gene were found in 11 hepatocellular carcinomas from Qidong, China, nor in four hepatocellular carcinomas experimentally induced by aflatoxin B_1. In humans the mutation and preferential binding of this aflatoxin to codon 249 occurs at the third nucleotide (guanine) while in duck, codon 249 lacks guanine; this could explain the differences in mutation spectra in the two species (Duflot *et al.*, 1994).

Experiments are continuing to compare carcinogen-metabolizing enzymes in chronically HBV-infected ducks and non-infected ducks.

2.11.4.3 *Comparative carcinogenicity*

Aflatoxin–serum albumin and aflatoxin–DNA adducts in the liver were measured in rats, hamsters, guinea-pigs and mice after multiple exposures to aflatoxin B_1. There was a fairly constant ratio between the two parameters across species, with adduct levels being highest in rat>guinea-pig>hamster>mouse. Aflatoxin–albumin adduct levels measured in human populations were similar for a given exposure to those seen in the rat (a species sensitive to aflatoxin carcinogenesis) and two orders of magnitude higher than those in the mouse (resistant to aflatoxin carcinogenesis).

Further experiments examined the relationship between aflatoxin–serum albumin adducts and genetic damage (micronuclei, chromosomal aberrations) in bone marrow in rats and mice. The level of chromosomal aberrations was about 10 times higher in rats than in mice. In rats both chromosomal aberrations and micronuclei showed positive correlations with aflatoxin-albumin adducts at the individual level (Anwar *et al.*, 1994).

2.11 IARC staff publications

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2.12 Cancer of the cervix

2.12.1 Case-control studies of cervical cancer in Spain and Colombia (p. 104 of 1992/93 Biennial Report)

The main results of these four case–control studies, published in 10 papers, confirm the central role of human papillomavirus (HPV) in the causation of both cervical intraepithelial neoplasia (CIN) III and invasive cervical cancer. Among the other sexually transmitted agents investigated, only infection with *Chlamydia trachomatis* was associated with a moderate increase in risk of CIN III, but it is not clear whether this represents a real association or reflects residual confounding due to undetected HPV DNA. An analysis based on the immunoglobulin G (IgG) subclasses of herpes simplex virus type 2 (IgG1 and IgG3) does not support an independent effect of this virus in cervical cancer. A preliminary analysis to explore the male role indicates that HPV DNA prevalence among controls' husbands is five

times higher in Colombia than in Spain. In Spain, both the number of partners of the husband and the HPV DNA prevalence are strong predictors of the risk among their wives. In Colombia, where the average number of partners and the HPV DNA prevalence are higher, an elevated risk associated with use of oral contraceptives was seen only when HPV DNA-positive cases were compared with HPV DNA-positive controls, suggesting that oral contraceptives may increase the risk of progression from the HPV DNA carrier state to CIN III and invasive cancer. A weak and inconsistent association was detected with tobacco smoking.

HPV serological assays have been carried out in collaboration with three laboratories. In the first study, antibodies to HPV 16 E6 and E7 proteins were measured in a sub-sample of cases and controls, using an assay which would detect antibodies to conformational epitopes. The results suggest that antibodies to HPV 16 E6 and E7 proteins are partially virus-specific and disease state-specific markers of HPV-associated cervical cancer (Viscidi *et al.*, 1993).

In the second study, sera from cases with invasive cancer and their controls were tested for the presence of antibodies to seven peptides derived from five open reading frames of HPV 16. Significantly higher prevalence of antibodies to E2 and E7 peptides was found in cases than in controls. The prevalence of antibodies to the three E7 peptides tested was higher and stronger in women positive for HPV 16 DNA than in women positive for other HPV DNA or in HPV DNA-negative women (Hamsiková *et al.*, 1994).

For the third study, serum from a sub-sample of cases and controls was tested for antibodies to HPV 16 virus-like particles using an ELISA assay. About half of the cases of invasive cancer were positive both in Spain and Colombia and 73% of CIN III cases in Spain and 81% of CIN III cases in Colombia. Among population controls, the antibody prevalence was 3% in Spain and 22% in Colombia, while for CIN III controls, the prevalence was 10% in Spain and 43% in Colombia. Correlation between the presence of HPV 16 DNA and positivity for antibodies was poor, suggesting that this assay may detect past as well as current HPV infection.

2.12.2 Multicentric case–control studies of cervical cancer (p. 107 of 1992/93 Biennial Report)

The results of the hospital-based case–control study carried out in Brazil confirm the central role of HPV and show an additional independent effect of hormonal factors (parity and oral contraceptives) among HPV DNA-positive women.

A preliminary analysis of the HPV DNA prevalence among husbands of cases and controls (42% and 48% respectively) did not show an association with the risk of cervical

cancer in their wives. A high background prevalence of HPV in the male population may preclude the detection of an association.

Data and specimen collection have been completed in Mali, Morocco, Paraguay, the Philippines and Thailand, as have most of the PCR-based HPV assays. Preliminary results again confirm the results of previous studies. The association with HPV is very strong in all studies, with odds ratios ranging from 21.3 in Paraguay to 97.7 in the Philippines. The prevalence of HPV DNA in exfoliated cells among cases ranged from 83.3% in Paraguay to 86% in the Philippines, and in biopsy specimens reached 95.1% in Morocco. Among controls, the HPV DNA prevalence ranged from 9.1% in the Philippines to 33.3% in Mali. However, as a high proportion of the specimens from Mali and Paraguay were β-globin-negative, the results should be interpreted with caution. In the Philippines, additional effects of tobacco smoking and parity among the HPV DNA-negative women were detected, but in Thailand no significant association with risk factors other than HPV was observed.

2.12. 3 International prevalence survey of HPV markers in cervical cancer tissue and sera (p. 108 of 1992/93 Biennial Report)

Over 1000 tumour tissue specimens from 22 countries were tested by PCR-based assays able to detect over 25 different HPV types, after a histological diagnosis of invasive cervical cancer had been confirmed. HPV DNA was detected in 93% of the tumours, with no significant variation in HPV positivity between countries. HPV 16 was present in 50% of the specimens, HPV 18 in 14%, HPV 45 in 8% and HPV 31 in 5%. HPV 16 was the predominant type in all countries but Indonesia, where HPV 18 was more common. There was significant geographical variation in the prevalence of some less common HPV types. A clustering of HPV 45 was apparent in western African, while HPVs 39 and 59 were virtually confined to Latin America. In squamous cell carcinomas, HPV 16 predominated, but HPV 18 predominated in adenocarcinomas and adenosquamous tumours (Bosch *et al.*, 1994). A new HPV type "IS39" (HPV 51-related) was identified in two specimens from Argentina and Cuba (Peyton *et al.*, 1994). HPVs 40, 42, 53, 54, 66 and PAP 155/MM8 were not found. HPVs 6, 11, 55 and PAP 291/MM7 each were found in only one or two specimens. Further analysis of HPV DNA-negative specimens is in progress. Overall, the results of this survey confirm the central etiological role of HPV in cervical cancer worldwide.

2.12.4 Studies of high-risk groups for cervical cancer in Spain and Colombia (p. 109 of 1992/93 Biennial Report)

As a complement to the case–control studies, the prevalence of CIN lesions was compared in a group of prostitutes and non-prostitutes from Spain and Colombia. No significant difference was found in the prevalence of CIN between the two countries, either among prostitutes (2.5% in Spain and 1.8% in Colombia) or among non-prostitutes (1.2% in Spain and 1.1% in Colombia). These results suggest that the eight-fold difference in risk of cervical cancer between the two countries is due to a higher prevalence in Colombia of factors acting in the progression from CIN to invasive cancer (de Sanjosé *et al.*, 1993).

2.12.5 Comparison of two sampling methods from the normal cervix in assessing HPV DNA prevalence

To assess the importance of the type of specimen in determining the HPV DNA prevalence in non-neoplastic uterine cervix, a validation study is being carried out in the Philippines, Thailand and Spain. Cervical scrapes and biopsies are being collected in 300 women with normal cytology in whom hysterectomy is performed for reasons other than cervical cancer. It is expected that the field work will be completed in 1995.

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2.13 Cancer of the breast

2.13.1 Breast cancer and reproductive and endocrine factors in Chinese premenopausal women (p. 111 of 1992/93 Biennial Report)

A case–control study was conducted in Guangzhou and risk factors similar to those already known in Caucasian populations were identified. Determinations of hormonal levels have been carried out recently and results will be incorporated in the analysis.

2.13.2 Case–control studies of selected second primary cancers following breast cancer and tamoxifen use (p. 112 of 1992/93 Biennial Report)

Tamoxifen is an antioestrogen which has been widely used in the treatment of breast cancer and more recently proposed for the prevention of breast cancer. However, the carcinogenic potential of tamoxifen warrants further epidemiological investigation before wide preventive use (Sasco, 1994a; Saez & Sasco, 1994). Preliminary results from a pilot study of endometrial cancer following breast cancer conducted in the Rhône and Côte d'Or show increased risk for long-term treatment. A multicentric case–control study is being set up and a study of the impact of tamoxifen on liver and ovarian cancer is now being planned.

2.13 IARC staff publications

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2.14 Other specific cancers and risk factors

2.14.1 Cancer of the pancreas, gallbladder and bile duct (p. 113 of 1992/93 Biennial Report)

Combined analyses of the data from the various centres are in progress.

2.14.2 Brain tumours in children (p. 114 of 1992/93 Biennial Report)

Data collection has been completed in all but one of the centres. Centre-specific analyses have been published for New South Wales (McCredie *et al.*, 1994a,b) and Milan, Varese and Como (Filippini *et al.*, 1994). In both of these studies, a positive association with passive exposure of the mother to tobacco smoke during the index pregnancy was found. In the study in New South Wales, the risk of childhood brain tumours increased with increasing consumption of cured meats during pregnancy, which is consistent with some other reports. In a preliminary combined analysis of data from five of the ten centres, there was a weak association with a family history of cancer. This was not related specifically to brain tumours or to any reported genetic syndromes. There was no association with reported congenital anomalies in the index child.

2.14.3 Brain tumours in adults (pp. 114–115 of 1992/93 Biennial Report)

Data collection has been completed in all ten centres, and centre-specific and combined analyses are in progress. In a preliminary combined analysis of data from seven centres, there was no evidence that glioma or meningioma occurring in adult life is associated with a family history of cancer or with a family history of epilepsy. Tuberous sclerosis and neurofibromatosis in either the index subject or his/her relatives was reported for less than 1% of cases.

2.14.4 Case–control study of plantar melanoma in Paraguay (p. 115 of 1992/93 Biennial Report)

Data analysis commenced in 1994. The file requires a considerable amount of checking and correction. Results should be available in 1995.

2.14.5 Case–control study of soft-tissue sarcoma and non-Hodgkin lymphoma in relation to exposure to herbicides in Viet Nam (pp. 115–116 of 1992/93 Biennial Report)

By mid-1994, 27 sarcoma cases, 46 lymphoma cases and 102 controls have been enrolled. Histological and immunological typing of the first 23 lymphoma cases has been carried out.

Preparation of exposure estimates by two independent methods for the first 100 cases has begun, in order to test the reproducibility of the methods.

2.14.6 Lung cancer in northern Thailand

The northern provinces of Thailand have a high incidence of lung cancer, particularly in women. A case–control study, including 200 lung cancer cases, 200 hospital controls and 200 community controls is investigating relevant etiological factors. The principal hypotheses include tobacco smoking, exposure to domestic smoke, and also exposure to environmental air pollution, since the area concerned contains many coal-fired electricity-generating stations. Recruitment should be completed during 1995.

2.14 IARC staff publications

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2.15 Role of cell–cell communication in carcinogenesis

2.15.1 Mutations of connexin genes (p. 124 of 1992/93 Biennial Report)

Our previous results have suggested that connexin (cx) 32 gene mutations in cancer are rare. One out of 13 rat liver tumours contained a cx 32 gene mutation, but none of 22 human gastric cancers and 20 liver tumours harboured any cx 32 gene mutation. On the other hand, there have been reports of cx 32 gene mutations in Charcot-Marie-Tooth syndrome (Bergoffen *et al.*, 1993; Fairweather *et al.*, 1994). We have examined the biological effect of

HeLa cells which lack gap junctional intercellular communication (GJIC). Initial results suggest that all the connexin genes mutated in regions other than C-terminal are non-functional, while mutant connexins lacking the C-terminal cytoplasmic region and the wild type restore GJIC function. We are now testing the hypothesis that mutant cx 32 may exert dominant negative regulation of wild-type cx 32 proteins by forming non-functional chimeric connexons between mutated and normal connexin molecules.

2.15.2 Mechanisms of aberrant localization of connexins in tumours (pp. 122–124 of 1992/93 Biennial Report)

Many human and rodent liver tumours show aberrant localization of connexin proteins. Certain tumour-promoting agents also induce aberrant localization of connexin proteins in rat liver (Krutovskikh *et al.*, 1994). We have begun to examine whether there exist one or more specific proteins responsible for transport of connexin proteins to cytoplasmic membranes; the transport function of such protein(s) might be disrupted in tumours or by tumour-promoting agents. Western blot analysis with cx 32 antibody does indeed suggest the presence of such a protein, usually present in conjunction with cx 32 protein. In addition, we have observed abnormal size and/or behaviour of this presumptive heterodimer of cx 32 and transporter protein in liver tumours.

The localization of connexin proteins is also possibly controlled by a cell adhesion molecule. When a mouse papilloma cell line which lacks E-cadherin was stained with cx 43 antibody, cx 43 protein was detectable only in the cytoplasm. However, when E-cadherin was transfected into this cell line (Jongen *et al.*, 1991), cx 43 was rapidly mobilized to the cytoplasmic membrane only after the culture was shifted to a high-calcium medium. Our preliminary results suggest that the intracellular transport and thus function of the connexin 43 protein is associated with its phosphorylation.

2.15.3 Tumour suppression and connexin expression (p. 121 of 1992/93 Biennial Report)

Transfection of cx 26, but not of cx 40 or cx 43, down-regulates the growth of HeLa cells *in vitro* and inhibits their tumorigenicity in nude mice (Mesnil *et al.*, 1994c). Analysis of various HeLa cx 26 transfectant subclones suggests that there is a correlation between the level of inhibition of cell growth and the amount of cx 26 protein expressed, as shown by immunocytochemistry. HeLa cells are derived from human cervical adenocarcinoma and we have observed that cx 26 is highly expressed in normal cervical tissue (Mesnil *et al.*, 1994b). These findings are compatible with the idea that cx 26 is a tumour-suppressor gene in HeLa

cells, and cx 26 has been suspected to act as a tumour-suppressor in other types of human cancers (Lee *et al.*, 1991; Grossman *et al.*, 1994).

In order to check whether connexin gene loci are involved in some chromosomal rearrangements or deletions, we have precisely located, through a collaboration with Dr M.-G. Mattei and Professor D. Gros (Marseille, France), the loci of the major connexins on the human genome as follows: cx 26 on 13q11-q12, cx 32 on Xq11-q12, and cx 43 on 6q21-q22. We are now studying correlations between loss of heterozygosity affecting these loci and human carcinomas.

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